on Rec'd PCT/PTO 19 JUN 2000

FORM PTO-1390 (REV 5-93) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY DOCKET NO. P108281-00000 DATE: June 19, 2000 U.S. APPLN. NO. (IF KNOWN, SEE 37 CFR 1.5) 09/581511
INTERNATIONAL APPLICATION NO. PCT/CA98/01184	INTERNATIONAL FILING DATE 18 December 1998	PRIORITY DATE CLAIMED 19 December 1997
TITLE OF INVENTION: HEMIASTERLIN ANALOGS		
APPLICANT(S) FOR DO/EO/US: Raymond ANDERSEN, Edward PIERS, James NIEMAN, John COLEMAN, Michel ROBERGE		

1. XX This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
   (THE BASIC FILING FEE IS ATTACHED)

2. _ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. XX This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT articles 22 and 39(1).

4. XX A proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. XX A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. XX is transmitted herewith (required only if not transmitted by the International Bureau).
   b. XX has been transmitted by the International Bureau.
   c. _ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. _ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. _ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. _ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. _ have been transmitted by the International Bureau.
   c. _ have not been made; however, the time limit for making such amendments has NOT expired.
   d. _ have not been made and will not be made.

8. _ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. _ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. _ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. XX An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. _ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. XX A FIRST preliminary amendment.
    _ A SECOND or SUBSEQUENT preliminary amendment.

14. _ A substitute specification.

15. _ A change of power of attorney and/or address letter.

16. XX Other items or information: PCT/IPEA/416, PCT/IPEA/409, PCT/IB/332, PCT/IPEA/402
    CHECK NO. 297723
    Drawings - 7 sheets

534 Rec'd PCT/PTO 19 JUN 2000

U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.50) 09/581511	INTERNATIONAL APPLICATION NO. PCT/CA98/01184	ATTORNEY DOCKET NO. P108281-00000 DATE: June 19, 2000	
17. XX The following fees are submitted: **Basic National Fee (37 CFR 1.492(a)(1)-(5):** Search Report has been prepared by the EPO or JPO....$840.00 International preliminary examination fee paid to USPTO (37 CFR 1.482)...$670.00 No international preliminary examination fee paid to USPTO (37 CFR1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))....$760.00 Neither international preliminary examination fee (37 CFR 1.482) or international search fee (37 CFR 1.445(a)(2)) paid to USPTO....$970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ....$ 96.00		CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =		$840	
Surcharge of $130.00 for furnishing the oath or declaration later than _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		$00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	21 - 20 =	01	X $ 18.00	$18	
Independent Claims	01 - 3 =	00	X $ 78.00	$00	
Multiple dependent claim(s) (if applicable)			+ $260.00	$00	
TOTAL OF ABOVE CALCULATIONS =				$858	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$00	
SUBTOTAL =				$858	
Processing fee of $130.00 for furnishing the English translation later the _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$00	
TOTAL NATIONAL FEE =				$858	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$00	
TOTAL FEES ENCLOSED =				$858	
				Amount to be refunded	$
				Charged	$

a. XX A check in the amount of $858 to cover the above fees is enclosed.
b. _ Please charge my Deposit Account No. 01-2300 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.
c. XX The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2300.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Arent Fox Kintner Plotkin & Kahn PLLC
1050 Connecticut Avenue, N.W., Suite 600
Washington, D.C. 20036
Telephone No. (202) 857-6000

Robert B. Murray
Reg. No. 22,980

09/581511
534 Rec'd PCT/PTO 19 JUN 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Raymond ANDERSEN et al

Serial No.: Unknown

Filed: June 19, 2000

For: HEMIASTERLIN ANALOGS

PRELIMINARY AMENDMENT

Commissioner of Patents
Washington, D.C. 20231 June 19, 2000

Sir:

Prior to calculation of the filing fee and prior to the examination of this application, please amend the above-identified application as follows:

IN THE CLAIMS:

Claim 4, line 1, delete ", 2 or 3".

Claims 5, 6 and 7, line 1 of each, delete "any of claims 1-4" and insert therefor --claim 1--.

Claims 8, 9 and 10, line 1 of each, delete "any of claims 1-7" and insert therefor --claim 1--.

Claims 12 and 14, line 1 of each, delete "any of claims 1-11" and insert therefor --claim 1--.

Claim 13, line 1, delete "any of claims 1-12" and insert therefor --claim 1--.

Claims 15 and 16, line 1 of each, delete "1-14" and insert therefor --claim 1--.

REMARKS

The above amendment to the claims has been made to correct the multiple dependency of the claims and to put the application in better condition for examination.

In the event that any fees are due in connection with this paper, please charge our Deposit Account No. 01-2300.

Respectfully submitted,
ARENT FOX KINTNER PLOTKIN & KAHN PLLC

Robert B. Murray
Attorney for Applicant
Reg. No. 22,980

Atty. Docket No.: P108281-00000
1050 Connecticut Avenue, N.W.
Suite 600
Washington, D.C. 20036
Tel (202) 638-5000
Fax (202) 638-4810

RBM/cb

7 /PRTS

09/581511

534 Rec'd PCT/PTC 19 JUN 2000

HEMIASTERLIN ANALOGS

Field of Invention

This invention relates to biologically active compounds and compositions, their use and derivation.

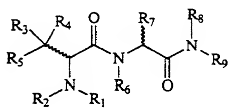
Background

As described in Talpir, R. et al. (1994) Tetrahedron Lett. 35:4453-6 and in international patent application PCT/GB96/00942 published October 24, 1996 under number WO96/33211, the compound hemiasterlin may be obtained from marine sponges or synthesized. As set forth in PCT/GB96/00942, hemiasterlin and the synthetic analogs described therein are cytotoxic and anti-mitotic.

Compounds that differ from hemiasterlin in the region of the indole moiety of hemiasterlin are novel. It has now been found that analogs of hemiasterlin wherein the indole moiety of hemiasterlin has been deleted or replaced demonstrate potent anti-mitotic and cytotoxic activity.

Summary of Invention

This invention provides a compound or pharmaceutically acceptable salt thereof, having the formula



I

wherein,

R_1 and R_2 are independently selected from the group consisting of: H, R, and ArR-, and where at least one of R_1 and R_2 is R and neither are ArR-, R_1 and R_2 together may optionally be a three to seven membered ring;

R_3 and R_4 are independently selected from the group consisting of: H, R, ArR-, and where at least one of R_3 and R_4 is R and neither are ArR- or Ar, R_3 and R_4 together may optionally be a three to seven membered ring;

R_5 is selected from the group consisting of: H, R, ArR-, and Ar;

R_6 is selected from the group consisting of: H, R, and ArR-;

R_7 and R_8 are independently selected from the group consisting of: H, R, and ArR-; and

R_9 is: $Z\text{-}\overset{O}{\overset{\|}{C}}\text{-}Y\text{-}$;

and wherein,

R is defined as a saturated or unsaturated moiety having a linear, branched, or cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, OH, $-OR_{10}$, $-O_2CR_{10}$, -SH, $-SR_{10}$, $-SOCR_{10}$, $-NH_2$, $-NHR_{10}$, $-N(R_{10})_2$, $-NHCOR_{10}$, $-NR_{10}COR_{10}$, -I, Br, -Cl, -F, -CN, $-CO_2H$, $-CO_2R_{10}$, -CHO, $-COR_{10}$, $-CONH_2$, $-CONHR_{10}$, $-CON(R_{10})_2$, -COSH, $-COSR_{10}$, $-NO_2$, $-SO_3H$, $-SOR_{10}$, $-SO_2R_{10}$, wherein R_{10} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

X is defined as a moiety selected from the group consisting of: -OH, -OR, =O, =S, $-O_2CR$, -SH, -SR, -SOCR,

$-NH_2$, $-NHR$, $-N(R)_2$, $-NHCOR$, $-NRCOR$, $-I$, $-Br$, $-Cl$, $-F$, $-CN$, $-CO_2H$, $-CO_2R$, $-CHO$, $-COR$, $-CONH_2$, $-CONHR$, $-CON(R)_2$, $-COSH$, $-COSR$, $-NO_2$, $-SO_3H$, $-SOR$, and $-SO_2R$;

Ar is defined as an aromatic ring selected from the group consisting of: phenyl, naphthyl, anthracyl, phenanthryl, furyl, pyrrolyl, thiophenyl, benzofuryl, benzothiophenyl, quinolyl, isoquinolyl, imidazolyl, thiazolyl, oxazolyl, and pyridyl, optionally substituted with R or X;

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R, ArR-, or X; and,

Z is defined as a moiety selected from the group consisting of: -OH, -OR; -SH; -SR; $-NH_2$; -NHR; $-N(R)_2$; $-NHCH(R_{11})COOH$; and $-NRCH(R_{11})COOH$, wherein R_{11} is a moiety having the formula: R, or $-(CH_2)_nNR_{12}R_{13}$, wherein n=1-4 and R_{12} and R_{13} are independently selected from the group consisting of: H; R; and $-C(NH)(NH_2)$.

This invention also provide methods of preparing the aforementioned compound of formula I, and precursors thereof, as described herein.

This invention also provides the use of the aforementioned compound of formula I, or a pharmaceutically acceptable salt thereof, for:

(a) manufacture of a medicament;

(b) in a method whereby cells, including tumor cells, which are susceptible to the cytotoxic effects of the compound are treated with the compound or a pharmaceutically acceptable salt thereof; and

(c) in a method whereby cells are treated with the compound or a pharmaceutically acceptable salt thereof, to bring about mitotic arrest in the cells, or the production of abnormal mitotic spindles in the cells.

Description of Figures

Figure 1 is a schematic showing a preferred scheme for synthesis of a compound of this invention.

Figure 2 is a schematic showing a preferred scheme for synthesis of the amino acid used in the coupling reaction shown in Figure 1.

Figure 3 is a schematic showing steps in the synthesis of a compound of this invention as described in the examples herein.

Figure 4 is a schematic showing steps in the synthesis of the dipeptide shown in Figure 3, as described in the examples herein.

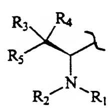
Figure 5A and 5B are graphs comparing the cytoxicity of hemiasterlin to SPA-110, as described in the examples herein.

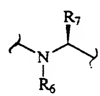
Figure 6 is a graph comparing the anti-mitotic activity of SPA-110 (◆) to hemiasterlin (□), as described in the examples herein.

Detailed Description of the Invention

Except where otherwise stated, the recitation of a compound herein covers all possible salts of the compound, and denotes all possible isomers possible within the structural formula given for such compound, including geometrical and optical isomers. Unless otherwise stated, materials described herein comprising a compound for which isomers exist, are to be regarded as covering individual isomers, and, mixtures of isomers including racemic mixtures.

In the compound of formula I set out above, bonds drawn in wavy line are from carbon atoms which may be optical centers. Preferably, the following absolute configurations predominate:





Except where otherwise stated, any moiety referred to herein which is described as "alkyl" will preferably be straight chain or, branched when possible, and will preferably have up to eight, more preferably up to six and even more preferably up to four carbon atoms. Except where otherwise stated optionally substituted alkyl groups are

preferably unsubstituted. Methyl is the most preferred alkyl group.

In this specification, reference is made to alkyl moieties being saturated or unsaturated, thereby including within the definition of the moiety, alkene and alkyne groups (whether internal, terminal or part of a ring).

In a compound of formula I, the following substituents alone, or in combination, are preferred:

(a) R_1 and R_2 independently: H, methyl, ethyl, propyl, n-butyl, acetyl; or, where R_1 and R_2 are joined: cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl; more preferably R_1 and R_2 are independently: H or CH_3; most preferably R_1 is H and R_2 is CH_3;

(b) preferably no more than one of R_3 and R_4 is H; more preferably, R_3 and R_4 are independently: methyl, ethyl, n-propyl or n-butyl, or, where R_3 and R_4 are joined: β-cyclopropyl, β-cyclobutyl, β-cyclopentyl or β-cyclohexyl; most preferably R_3 and R_4 are each methyl;

(c) R_5: Ar in the definition of R_5 is preferably phenyl, naphthyl, anthracyl or pyrrolyl; preferably R_5 is phenyl, methyl or H; most preferably R^5 is phenyl or methyl;

(d) R_6 and R_8 independently: H or methyl, more preferably R_6 is H and R_8 is methyl;

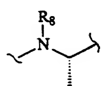
(e) R_7: a three to six carbon, branched alkyl group; more preferably R_7 is $-C(CH_3)_3$; and

(f) in R_9, Z is preferably OH, $-OR_{14}$ (wherein R_{14} is a linear or branched one to six carbon alkyl group, $-NHCH(R)_{11})COOH$ or $-NCH_3CH(R_{11})COOH$ wherein R_{11} is R, or $-(CH_2)_nNHC(NH)(NH_2)$; or R_9 is preferably

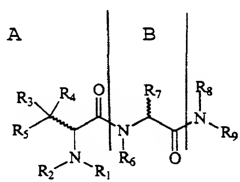
$-\underset{R_{15}}{C}-C=C(R_{16})\overset{O}{\overset{\|}{C}}-OH$ wherein R_{15} is methyl, ethyl, n-propyl, isopropyl, tert-butyl, iso-butyl, or sec-butyl and R_{16} is H, methyl, ethyl, propyl, iso-propyl, n-butyl, iso-butyl or sec-butyl; more preferably Z is OH and R_9 as a whole is:

$$-\underset{CH(CH_3)_2}{C}-C=C(CH_3)\overset{O}{\overset{\|}{C}}OH$$

Where R_9 has a chiral center in moiety Y, the following absolute configuration is preferred, with reference to an example where the chiral center has a methyl substituent:



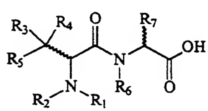
Compounds of formula I may be prepared by coupling moieties A, B and C as represented below using standard procedures, including procedures for coupling amino acids through a peptide bond.



A coupling agent, for example PyBroP, is suitably used in the reaction. The reaction suitably comprises connecting amino acid moieties in the presence of the coupling agent, a base such as 4-dimethylaminopyridine and an organic solvent such as methylene chloride. Standard reaction quenching and purification procedures known in the art produce the coupled compound.

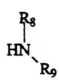
Preparation of moieties B and C as described above may be carried out using procedures and starting materials known in the art, for example by following the methods described in the aforementioned PCT/GB96/00942. The methods as set out in the examples herein may be employed, with suitable modification to materials and reagents, according to particular substituents of moieties A, B and C.

One aspect of this invention is a method for preparing a compound of formula I in which a compound of the formula:



II

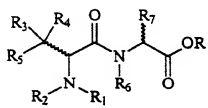
is coupled with a compound of the formula:



III

A compound of formula III may be prepared by known methods (such as described in PCT/GB96/00942) and by the method described in the examples herein.

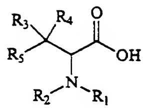
A further aspect of this invention is a method for preparing a compound of the formula II described above in which a compound of the formula:



IV

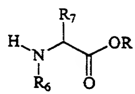
is treated with a base such as dilute sodium hydroxide in a solvent such as methanol for enough time to allow removal of OR; followed by acidification to about pH 3. Preferably R in formula IV is a simple alkyl chain, such as CH_3, and a protecting agent such as tert-butoxycarbonyl (Boc) may be used to protect the amine group; i.e. R_1 or R_2 is replaced by Boc. The Boc group is suitably removed by a reaction such as TFA/CH_2Cl_2 for about 1 hour at ambient temperatures. An appropriate isolation protocol produces the TFA salt. Subsequently another group (eg. R_1 or R_2) could be introduced on the nitrogen by standard techniques known to any person in the art, which produces compound of formula IV.

A further aspect of this invention is a method for preparing a compound of formula IV described above in which a compound of the formula:



V

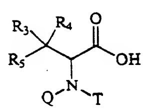
is coupled with a compound of the formula:



VI

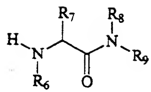
Preparation of compound V can be accomplished by many procedures known to person skilled in the art. One such example is described below in Figure 2. Compounds of formula VI may be prepared by methods known to persons of skill in the art.

A preferred method according to this invention for preparing a compound of formula I, is to prepare a dipeptide comprising moieties B and C and couple the dipeptide to moiety A. In this method, a compound of the following formula, wherein Q and T together are a combination of any two of the substituents: R_1, R_2 and a protecting group:



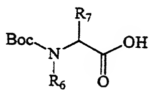
VII

is coupled with a compound of the formula:



VIII

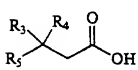
A compound of formula VIII may be prepared by coupling a compound of formula III as described above, with a compound of the formula:



IX

Compounds of formula IX may be prepared by methods known to persons of skill in the art.

A further aspect of this invention is a method to prepare a compound of formula VII as described above in which a compound of the formula:



X

is treated with a base followed by an azide compound. The azide derivative so produced is reduced to form an amine which is then treated with groups selected from: R_1, R_1 and Boc in the presence of a base such as sodium hydride.

Figure 1 sets out a preferred scheme for preparation of a compound of formula I involving the coupling of amino acid moiety A with a dipeptide comprising moieties B and C. In the embodiment shown in the figure, the substituents of the dipeptide are those of hemiasterlin. The Boc protected dipeptide portrayed in the figure may be obtained by the methods set out in the examples herein. On the A moiety, Boc may replace R_1 rather than R_2 or both R_1 and R_2 may be present on the A moiety prior to coupling.

Figure 2 sets out a preferred scheme for preparation of moiety A as used in the scheme shown in Figure 1. R_2 may be added in place of Boc and Boc may replace R_1.

Compounds of formula I are biologically active. The invention includes the use of a compound of formula I. Compounds of formula I may have pesticidal, for example insecticidal activity. Preferably, however, the use is in the pharmaceutical field for medical or veterinarial applications.

The compounds described herein have utility as cytotoxic agents, particularly against tumor cells and may have utility as anti-bacterial or anti-viral agents. Therefore, this invention includes a pharmaceutical composition comprising an effective amount of a compound of formula I, in association with a carrier.

This invention further provides the use of a compound of formula I for the manufacture of a medicament for use in the treatment of cancer or a tumor in a mammal.

In using a compound of formula I for medical or veterinarial applications, the compound is preferably administered in a pharmaceutical composition comprising also a pharmaceutically acceptable carrier, and optionally, one or more other biologically active ingredients. Such compositions may be in any form used for administering pharmaceuticals, for example any form suitable for oral, topical, vaginal, intravenous, subcutaneous, parenteral, rectal and inhalatory application. The compositions may be provided in discrete dose units. The carriers may be particulate, with the compositions being, for example, tablets or powders, or liquid, with the compositions being, for example, oral syrups or injectable liquids, or aerosol, for inhalatory application.

For oral administration an excipient and/or binder may be present. Examples are sucrose, kaolin, glycerin, starch dextrins, sodium alginate, carbosymethylcellulose and ethyl cellulose. Colouring and/or flavouring agents may be present. A coating shell may be employed. For rectal administration oleaginous bases may be employed, for example lanolin or cocoa butter. For an injectable formulation buffers, stabilizers and isotonic agents may be included.

The dosage of a compound of formula I may depend upon the weight and physical condition of the patient; on the severity and longevity of the illness; and on the particular form of the active ingredient, the manner of administration and the composition employed. A daily dose of about 0.0001 to about 100 mg/kg of body weight taken singly or in separate doses of up to 6 times a day, or by a continuous infusion, embraces the effective amounts most typically required. A preferred range is about 0.001 to about 50 mg/kg of body weight, per day, most preferably about 0.01 to about 30 mg/kg of body weight, per day.

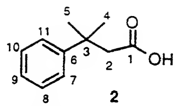
It is to be understood that use of a compound of formula I in chemotherapy can involve such a compound being bound to an agent, for example a monoclonal or polyclonal antibody, a protein or a liposome, which assists the delivery of the said compound to tumor cells.

This invention also includes the use of a compound of formula I as an antimitotic agent. Such use may be in procedures that require blocking cells in mitosis, such as the preparation of mitotic spreads for karyotype analysis. The compounds of this invention can also be used to probe microtubule function in mitotic cells.

Examples

The following examples provide a detailed description of preferred methods of synthesis of a preferred compound of this invention, SPA-110. Also described are precursor compounds and characterization of various compounds of this invention. Figures 3 and 4 schematically portray the synthesis of the a SPA-110 salt according to the examples. Reference numerals in the examples correspond to labelling of compounds in Figures 3 and 4 and labelling of compounds depicted in the examples.

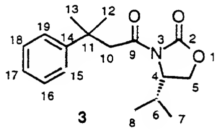
3-methyl-3-phenylbutanoic acid (2)



2

3-Methyl-2-butenoic acid (**1**, 5.10 g, 50.9 mmol) and $AlCl_3$ (20.4 g, 153 mmol) were placed in a one-neck round-bottomed flask. Benzene (50 mL) was added, which produced vigorous bubbling. Upon completion of the bubbling, a capped condenser (*i.e.* closed system) was attached, the reaction mixture was stirred and placed in an oil bath at 65°C. The pressure in the system was occasionally released. The progress of the reaction was followed by following the loss of starting material by GC. If the reation was not complete within 1 h, a small quantity of $AlCl_3$ was added and stirring was continued. To the solution was added diethyl ether and the mixture was cooled to 0°C. Slowly conc. HCl and some water were added until all the solid dissolved and the pH was less than 2. The aqueous layer was extracted with diethyl ether three times. The organic layer was concentrated to 150 mL and then was extracted with a saturated sodium hydrogen carbonate solution six times. The combined aqueous layer were acidified with conc. HCl until the pH was less than 2. The acidic aqueous layer was extracted with diethyl ether three times and the accumulated organic layer was dried with magnesium sulfate. The solution was filtered and the diethyl ether was removed *in vacuo* producing a white solid (8.51 g, 47.7 mmol) in 94% yield, which did not need further purification. mp 55 - 56°C. ^{1}H-NMR (400 MHz, $CDCl_3$) 10.45 (bs, 1H, $CO_2\underline{H}$), 7.38 (d, 2H, J = 7.2 Hz, H-11 and H-7), 7.32 (t, 2H, J = 7.2 Hz, H-10 and H-8), 7.21 (t, 2H, J = 7.2 Hz, H-9), 2.65 (s, 2H, H-2), 1.47 (s, 6H, H-5 and H-4); Mass spectrum (EI) 178 (23, $M^{\cdot+}$), 119 (100, $[C_9H_{11}]^+$). For pioneering work to form **2** see: F.J. Eijkman (1908) Chem. Kentr. II, p. 110; or A. Hoffman (1929) J. Am. Chem. Soc. 51:2542.

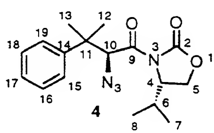
(4*S*)-3-(3-methyl-3-phenyl-1-oxobutyl)-4-isopropyl-2-oxazolidinone (3)



3

3-Methyl-3-phenylbutanoic acid (**2**, 1.00 g, 5.61 mmol) was dissolved in 70 mL of THF and cooled to -78°C. Triethylamine (1.17 mL, 8.42 mmol) and trimethylacetyl chloride (0.760 mL, 6.17 mmol) were added to the reaction flask producing a white solid. The resulting mixture was warmed to 0°C for 1 h and then cooled back down to -78°C. In a second flask butyllithium (6.84 mL, 1.6 *M* in hexanes, 10.9 mmol) was added dropwise with vigorous stirring to a solution of (4*S*)-(-)-4-isopropyl-2-oxazolidinone (1.45 g, 11.2 mmol) at -78°C in THF (60 mL) producing a white precipitate. The resulting suspension of the lithiated oxazolidinone was added *via* cannula to the reaction flask. Stirring was continued for 2 h, water was added and the reaction mixture was warmed to room temperature, whereupon it was extracted three times with diethyl ether. The combined organic extracts were dried over magnesium sulfate, and concentrated *in vacuo*. The product was purified by radial chromatography (4 mm plate, 3 : 7 diethyl ether - pet. ether) affording compound **3** as a clear, colourless oil in 84% yield (1.37 g, 4.74 mmol). ^{1}H-NMR (400 MHz, $CDCl_3$) 7.38 (d, 2H, J = 7.3 Hz, H-19 and H-15) 7.28 (t, 2H, J = 7.3 Hz, H-18 and H-16), 7.16 (t, 1H, J = 7.3 Hz, H-17), 4.22 - 4.18 (m, 1H, H-4), 4.05 (dd, 1H, J = 9.0 and 2.8 Hz, 1H-5), 4.00 (t, 1H, J = 9.0 Hz, 1H-5), 3.38 - 3.30 (m, 2H, H-10), 2.16 - 2.12 (m, 1H, H-6), 1.48 (s, 3H, H-13 or H-12), 1.47 (s, 3H, H-13 or H-12), 0.79 (d, 3H, J = 7.1 Hz, H-8 or H-7), 0.71 (d, 3H, J = 6.9 Hz, H-8 or H-7); Mass spectrum (EI) 289 (8, $M^{\cdot +}$), 119 (100, $[C_9H_{11}]^+$). Optical rotation obtained was $[\alpha]_D^{25}$ +69.5 (c 1.16, $CHCl_3$). Compound 3 was prepared according to D.A. Evans et al. (1988) Tetrahedron 44:5525.

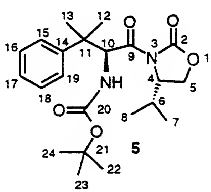
Preparation of the 4-isopropyl-2-oxazolidinone 4



4

Oxazolidinone 3 (472 mg, 1.63 mmol), dried under high vacuum for 0.5 h, was dissolved in THF and cooled to -78°C (10 mL). Freshly titrated potassium bis(trimethylsilyl)amide (15.6 mL, 0.115 *M* in THF, 1.79 mmol) was added and the resulting solution was stirred at -78°C for 1 h. A solution of 2,4,6-triisopropylbenzenesulfonyl azide (625 mg, 2.04 mmol) in THF (5 mL) at -78°C was added *via* cannula and after 2 min. the orange coloured reaction mixture was treated with glacial acetic acid (0.429 mL, 7.50 mmol), warmed to 40°C in a water bath and stirred for a further hour. To the light yellow mixture was added brine (35 mL), water (35 mL) and the aqueous phase was extracted three times with 80 mL diethyl ether. The combined organic extracts were washed with a saturated sodium hydrogen carbonate solution (20 mL), dried with magnesium sulfate and concentrated *in vacuo*. The product was purified by radial chromatography (4 mm plate, 3 : 7 diethyl ether - pet. ether, sample was loaded with diethyl ether) affording azide 5 as a colourless oil (482 mg, 1.46 mmol) in 89% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.39 (d, 2H, J = 7.2 Hz, H-19 and H-15), 7.31 (t, 2H, J = 7.2 Hz, H-18 and H-16), 7.23 (t, 1H, J = 7.2 Hz, H-17), 5.64 (s, 1H, H-10), 3.95 (dd, 1H, J = 8.7 and 2.2 Hz, 1H-5), 3.89 - 3.85 (m, 1H, H-4), 3.56 (t, 1H, J = 8.7 Hz, 1H-5), 2.31 - 2.26 (m, 1H, H-6), 1.54, 1.52 (s, 3H, H-13 and H-12), 0.83 (d, 3H, J = 7.0 Hz, H-8 or H-7), 0.79 (d, 3H, J = 6.9 Hz, H-8 or H-7); Mass spectrum (DCI, NH_3) 349 (45, $[M+NH_5]^+$), 348 (100, $[M+NH_4]^+$), 331 (12, $[M+H]^+$), 303 (57, $[M-N_2]^+$), 119 (94, $[C_9H_{11}]^+$). Optical rotation obtained was $[\alpha]_D^{25}$ +121.5 (c 1.1, $CHCl_3$). Compound 4 was prepared according to the methodology developed by D.A. Evans et al. (1990) J. Am. Chem. Soc. 112: 4011. 2,4,6-Triisopropylbenzenesulfonyl azide was prepared by the method of O.C. Dermer et al. (1955) J. Am. Chem. Soc. 77:70.

Preparation of the 4-isopropyl-2-oxazolidinone 5

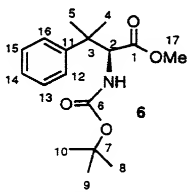


5

Azide 4 (418 mg, 1.26 mmol), 10% palladium on charcoal (280 mg), and di-*tert*-butyl dicarbonate (608 mg, 2.78 mmol) were placed in a 100 mL flask. Ethyl acetate (37 mL) was added and the resulting black suspension was stirred at room temperature. The mixture was

flushed with argon, then with hydrogen and was stirred under a hydrogen balloon overnight (~14 h). The reaction mixture was filtered through silica gel and the collected material was washed with ethyl acetate. The combined filtrate was concentrated *in vacuo* and the crude mixture was purified by flash column chromatography (3 : 7 diethyl ether - pet. ether) to afford compound **5**, a viscous colourless oil, in 78% yield (400 mg, 0.989 mmol). ^{1}H-NMR (400 MHz, $CDCl_3$) 7.40 (d, 2H, J = 7.4 Hz, H-19 and H-15), 7.29 (t, 2H, J = 7.4 Hz, H-18 and H-16), 7.21 (t, 1H, J = 7.4 Hz, H-17), 6.12 (d, 1H, J = 9.9 Hz, H-10), 5.11 (bs, 1H, N-H), 3.89 (d, 1H, J = 8.4 and 1.9 Hz, H-5), 3.82 - 3.79 (m, 1H, H-4), 3.45 (t, 1H, J = 8.4 Hz, H-5), 2.26 - 2.22 (m, 1H, H-6), 1.41 (s, 9H, H-24,H-23 and H-22), 0.80 (d, 3H, J = 7.0 Hz, H-8 or H-7), 0.76 (d, 3H, J = 6.9 Hz, H-8 or H-7); Mass spectrum (DCI, CH_4/NH_3 mix) 405 (1, $[M+H]^+$), 349 (7, $[M-C_4H_9]^+$), 230 (100, $[C_9H_{14}N_2O_5]^+$). Optical rotation obtained was $[\alpha]_D^{24}$ +118.4 (c 0.935, $CHCl_3$). Compound **5** was prepared according to the methodology developed by D.A. Evans et al. (1990) [supra].

Methyl (2*S*)-2-(*tert*-butyloxycarbonyl)amino-3-methyl-3-phenylbutanoate (**6**)

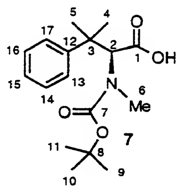


6

Oxazolidinone **5** (245 mg, 0.605 mmol) was dissolved in a mixture of 7.1 mL THF and 1.8 mL water. This solution was cooled to 0°C and hydrogen peroxide (0.618 mL, 30% aqueous, 5.45 mmol) and lithium hydroxide (1.82 mL, 1.0 *M*, 1.82 mmol) were added. The resulting mixture was stirred at room temperature overnight (~15 h). The excess peroxide was quenched by addition of sodium hydrogen sulfite (7.1 mL, 1.5 *M*, 10.7 mmol) and stirring was continued for 1 h. The aqueous phase was acidified with 1.0 *M* citric acid and the mixture was extracted three times with ethyl acetate. The combined ethyl acetate extracts were dried over magnesium sulfate and concentrated *in vacuo*. To the remaining crude material was added a solution of diazomethane in diethyl ether until the solution stayed yellow. After bubbling argon through the solution for 15 min., the remaining volatile components were removed *in vacuo* to afford crude compound **6**. Purification of ester **6** was accomplished by radial chromatography (2 mm plate, 3 : 7 diethyl ether - pet. ether, sample was loaded with $CHCl_3$), producing a clear colourless oil (171 mg, 0.555) in

92% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) 7.33 - 7.27 (m, 4H, H-16, H-15, H-13, H-12), 7.20 (t, 1H, J = 6.7 Hz, H-14), 4.99 (bd, 1H, J = 8.8 Hz, H-2), 4.50 (bd, 1H, J = 8.8 Hz, N-H), 3.48 (s, 3H, H-17), 1.41, 1.38 (s, 3H, H-5 and H-4), 1.37 (s, 9H, H-10,H-9, and H-8); Mass spectrum (EI) 307 (0.1, $M\cdot^+$), 234 (2, $[M\text{-}Ot\text{-}Bu]^+$), 119 (100, $[C_9H_{11}]^+$). Optical rotation obtained was $[\alpha]_D^{25}$ +35.2 (c 2.98, $CHCl_3$). Compound **5** was prepared according to the methodology developed by D.A. Evans et al. (1990) [supra].

(2*S*)-*N*-*tert*-butoxycarbonyl-*N*-methyl-3-methyl-3-phenylbutanoic acid (7)

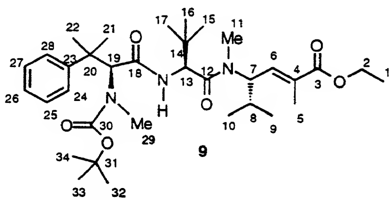


7

To a vigorously stirred solution of ester **6** (43.4 mg, 0.141 mmol) in 2 mL dry DMF were added sodium hydride (10.2 mg, 4.24 mmol) followed by methyl iodide (0.088 mL, 1.41 mmol) and the resulting grey suspension was stirred overnight (~20 h) at room temperature. The excess sodium hydride was quenched by cautious addition of water and the mixture was acidified by dropwise addition of 1.0 *M* citric acid. The acidic mixture was extracted three times with ethyl acetate, the combined organic layer extracted three times with brine, dried over magnesium sulfate and concentrated *in vacuo*. The resulting light orange oil was dissolved in 4 mL methanol in a 25 mL flask. To the solution was added 1.0 mL of water, followed by 1.13 mL of 1.0 *M* lithium hydroxide. The reaction mixture was heated at 60°C overnight (~14 h), producing a white precipitate. To the resultant mixture was added saturated sodium hydrogen carbonate solution and water; the mixture was then extracted with ethyl acetate. The aqueous layer was acidified with 1.0 *M* citric acid until the pH was ~ 4. The mixture was extracted three times with ethyl acetate. The combined organic layers were dried with magnesium sulfate and concentrated *in vacuo*. Compound **7** was also found in the first ethyl acetate extraction so it was also added to the crude product. Purification of acid **7** was performed by silica gel column chromatography (1 : 2 diethyl ether - pet. ether with 1% acetic acid) resulting in a 49% yield (21.2 mg, 0.0670 mmol) of a clear colourless oil . ^{1}H-NMR (400 MHz, $CDCl_3$) 7.41 (d, 1.3H, J = 7.6 Hz, H -17 and H -13), 7.37 (d, 1.3H, J = 7.6 Hz, H -17 and H -13), 7.28 (t, 2H, J = 7.6 Hz, H-16 and H-14), 7.18 (t, 1H, J

= 7.2 Hz, H-15), 5.17 (bs, 0.66H, H-2), 4.93 (bs, 0.33H, H-2), 2.75 (s, 1.05H, H-6), 2.62 (s, 1.95H, H-6), 1.55 (s, 3H, H-5 or H-4), 1.49 - 1.39 (m, 12H, H-5 or H-4 and H-11, H-10 and H-9); Mass spectrum (EI) 307 (0.1, M·+), 234 (3, [M-O*t*-Bu]+), 119 (100, $[C_9H_{11}]^+$), 57 (78, $[C_4H_9]^+$); Exact mass calc d for $C_{17}H_{25}NO_4$: 307.1783. Found (EI): 307.1793.

Preparation of Compound 9

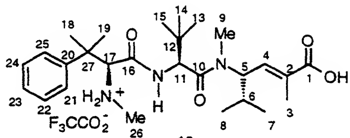


9

The *N*-Boc-amino ester **8** (71.6 mg, 0.174 mmol) was dissolved in 1 mL CH_2Cl_2 and 1 mL of TFA was added. The reaction mixture was stirred at room temperature for 0.5 h. Removal of the solvent *in vacuo*, followed by repeated rinsing of the remaining material with CH_2Cl_2 (3 x 5 mL) and evaporation of the residual solvent afforded the TFA salt of the amino acid ester **8**. In a separate flask, to a solution (or suspension) of the *N*-Boc protected amino acid **7** (51.5 mg, 0.167 mmol) in 0.5 mL CH_2Cl_2, was added DIEA (0.0875 mL, 0503 mmol), DMAP (0.031 mg, 0.10 mmol) and PyBroP (0.0781 mg, 0.167 mmol). The solution was stirred for a few minutes and then a solution of the TFA salt of **8** was added in 1 mL of CH_2Cl_2 *via* cannula addition. The reaction mixture was stirred at room temperature for 18 h. To the mixture was added water, CH_2Cl_2 and ten drops of 10% aqueous HCl. The resulting biphasic solution was extracted with CH_2Cl_2 (three times with 20 mL). The organic layer was extracted with saturated aqueous sodium hydrogen carbonate (10 mL), dried with magnesium sulfate and the solvent was removed *in vacuo*. The product was purified by flash chromatography (silica gel, 1 : 1 diethyl ether - pet. ether) affording the protected tripeptide **9** as a clear colourless oil in 27% yield (0.0272 g, 0.0454 mmol). ^{1}H-NMR (400 MHz, $CDCl_3$) 7.84 (bd, 1H, J = 9.5 Hz, N-H), 7.4- -7.30 (m, 5H, H-28, H-27, H-25, H-24), 7.21 (bt, 1H, J = 7.2 Hz, H-26), 6.63 (bd, 1H, J = 9.6 Hz, H-6), 5.08 (t, 1H, J = 9.6 Hz, H-7), 4.83 (d, 1H, J = 9.5 Hz, H-13), 4.17 (q, 2H, J = 7.1 Hz, H-2), 3.02 (s, 3H, H-11), 2.15 (s, 0.66H, H-29), 2.02 (s, 2.37H, H-29), 1.94 - 1.81 (m, 1H, H-8), 1.88 (s, 3H, H-5), 1.39 - 1.38 (m, 9H, H-34, H-33 and H-32), 1.28 (t, 3H, J = 7.1 Hz, H-1), 0.98 (s, 9H, H-

17, H-16 and H-15), 0.83, 0.77 (d, 3H, J = 6.6 Hz, H-10 and H-9); PyBroP is described in E. Frérot et al. (1991) Tetrahedron 47:259.

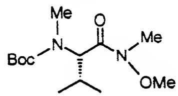
SPA110 - trifluoroacetate salt (**10**)



10

To a solution of the ethyl ester **9** (23.0 mg, 0.0382 mmol) in 1.1 mL MeOH was added 0.30 mL water and 0.31 mL of a 1.0 *M* aqueous solution of lithium hydroxide (0.31 mmol). The reaction mixture was stirred at room temperature overnight (~20 h) whereupon it was acidified by dropwise addition of 1.0 *M* citric acid and then extracted three times with ethyl acetate. The combined organic extracts were dried with magnesium sulfate and concentrated *in vacuo*. Under an argon atmosphere, the crude oil was dissolved in 1 mL CH_2Cl_2 and the solution was treated with TFA (1 mL) and then was stirred at room temperature for 0.5 h. Removal of the excess solvents *in vacuo*, followed by rinsing of the remaining material three times with CH_2Cl_2 (5 mL) and evaporation of the residual solvent, produced the TFA salt. HPLC purification of the crude product using a Magnum reverse phase C-18 column (H_2O(45) : MeOH(55) with 0.05% TFA) afforded the tripeptide **10** as a white powder. ^{1}H-NMR (400 MHz, CD_3OD) 7.53 (d, 2H, J = 7.6, H-25 and H-21), 7.44 (t, 2H, J = 7.6 Hz, H-24 and H-22), 7.34 (t, 1H, J = 7.6 Hz, H-23), 6.76 (d, 1H, J = 9.1 Hz, H-4), 5.04 (t, 1H, J = 10.1 Hz, H-5), 4.91, 4.34 (s, 1H, H-17 and H-11), 3.13 (s, 3H, H-9), 2.49 (H-26), 2.08 - 1.99 (m, 1H, H-6), 1.90 (s, 3H, H-3), 1.46, 1.37 (s, 3H, H-19 and H-18), 1.05 (s, 9H, H-15, H-14 and H-13), 0.89 (d, 3H, J = 6.1 Hz, H-8 or H-7), 0.88 (d, 3H, J = 6.5 Hz, H-8 or H-7); Mass spectrum (EI) 474 (0.1, [M-$CF_3CO_2^-$]$^+$), 458 (0.1, [M-16-$CF_3CO_2^-$]$^+$), 382 (2), 162 (62), 69 (74), 45(100).

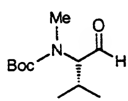
N^{α}-Boc-N^{α}-methyl-l-valine *N*-methoxy-*N*-methylamide (12)



12

To a cold (0°C) solution of *N*-Boc-*N*-methylvaline (**11**) (5.0 g, 21.6 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (2.8 g, 28 mmol), and PyBOP® (11.2 g, 22 mmol) in CH_2Cl_2 (22 mL) was added DIEA (8.4 mL, 75 mmol). After 1 min., the reaction mixture was warmed to room temperature and stirring was continued for 1 h. If the pH value of the mixture was less than 7, the mixture could be treated with a few drops DIEA to allow the reaction to go to completion. The mixture was poured into 200 mL of diethyl ether and the resultant mixture was washed successively with 3 N hydrochloric acid (3 x 30 mL), saturated aqueous sodium hydrogen carbonate solution (3 x 30 mL), and saturated aqueous sodium chloride (3 x 30 mL). The organic layer was dried with magnesium sulfate and the solvent was evaporated, followed by chromatography of the crude product (silica gel, 1 : 3 diethyl ether - pet. ether), afforded **12** (4.46 g, 75% yield) as a colourless oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.84 (d, J = 6.6 Hz, 4H, $(CH_3)_2$), 0.85 (d, J = 6.6 Hz, 2H, $(CH_3)_2$), 1.41 (s, 6H, Boc-$(CH_3)_3$), 1.44 (s, 3H, Boc-$(CH_3)_3$), 2.15 - 2.30 (m, 1H, CH), 2.75 (s, 1H, NCH_3), 2.78 (s, 2H, NCH_3), 3.10 (bs, 3H, NCH_3), 3.64 (s, 1H, OCH_3), 3.68 (s, 2H, OCH_3), 4.66 (d, J = 10 Hz, 0.4H, CH), 4.95 (d, J = 10 Hz, 0.6H, CH); Exact mass calc'd for $C_{13}H_{27}N_2O_4$ $(M+H)^+$: 275.19708. Found (DCI): 275.19710. Optical rotation obtained was $[\alpha]_D^{25}$ +128.3 (c 2.9, $CHCl_3$).

N-Boc-*N*-methyl-l-valinal (13)

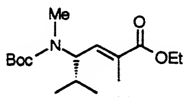


13

Lithium aluminum hydride (875 mg, 23 mmol) was added to a solution of N^{α}-Boc-N^{α}-methyl-*L*-valine *N*-methoxy-*N*-methylamide (**12**) (2.0 g, 7.7 mmol) in dry THF (8 mL) and the reaction mixture was stirred for 20 min. The mixture was poured into a stirring solution of potassium hydrogen sulfate (3.14 g, 23 mmol) in water (100 mL). Diethyl ether (75 mL) was

added, the layers separated and the aqueous layer extracted with diethyl ether (3 x 50 mL). The organic layers were combined, and washed sequentially with 3 N hydrochloric acid (3 x 30 mL), saturated aqueous sodium hydrogen carbonate (3 x 30 mL), and saturated aqueous sodium chloride (3 x 30 mL). The organic layer was dried with magnesium sulfate and the solvent was evaporated to yield the crude aldehyde **13** (1.52 g, 92% yield). Aldehyde **13** was used without further purification. Note: **13** can be stored under argon for ~ 2 weeks, but when stored in organic solvents at room temperature, undergoes slow decomposition. ^{1}H-NMR (200 MHz, $CDCl_3$) 0.73 (d, J = 6.9 Hz, 3H, CH_3), 0.91 (d, J = 6.9 Hz, 3H, CH_3), 1.27 (s, 9H, Boc-$(CH_3)_3$), 2.02 - 2.15 (m, 1H, CH), 2.63 (s, 2H, NCH_3), 2.72 (s, 1H, NCH_3), 3.44 (d, J = 9.5 Hz, 0.5H, CH), 3.86 (d, J = 9 Hz, 0.5H, CH), 9.45 (s, 1H, CH); Exact mass calc'd for $C_{11}H_{22}NO_3$ $(M+H)^+$: 216.15997; Found (DCI): 216.15996; Optical rotation obtained was $[\alpha]_D^{25}$ -104.2 (c 5.5, $CHCl_3$).

Ethyl (2*E*,4*S*)-*N*-Boc-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (14)



14

To a solution of aldehyde **13** (1.75 g, 8.7 mmol) in dry CH_2Cl_2 (9.0 mL) under an argon atmosphere at room temperature was added (carbethoxyethylidene)triphenylphosphorane (4.19 g, 11.3 mmol) and stirring was continued for a 4 h. The reaction mixture was diluted with water (100 mL) and extracted with diethyl ether (3 x 100 mL). The combined organic extracts were washed with saturated aqueous sodium chloride (100 mL), dried with magnesium sulfate and concentrated *in vacuo*. The crude oil was purified by flash chromatography (silica gel, 2 : 23 diethyl ether - pet. ether) to afford the required *E*-2-alkenoate **14** as a colourless oil (2.13 g, 82% yield). ^{1}H-NMR (200 MHz, $CDCl_3$) 0.74 (d, J = 6 Hz, 3H, CH_3), 0.79 (d, J = 6 Hz, 3H, CH_3), 1.17 (t, J = 7 Hz, 3H, CH_3), 1.34 (s, 9H, Boc-$(CH_3)_3$), 1.72 (m, 1H, CH), 1.78 (s, 3H, CH_3), 2.60 (bs, 3H, NCH_3), 4.08 (q, J = 7 Hz, 2H, CH_2), 4.15 - 4.20 (m, 0.5H, CH), 4.21 - 4.32 (m, 0.5H, CH), 6.54 (d, J = 8 Hz, 1H, CH); Exact mass calc'd for $C_{16}H_{30}NO_4$ $(M+H)^+$: 300.21750; Found (DCI): 300.21754. Optical rotation obtained was $[\alpha]_D^{25}$ +61.1 (c 9.1, $CHCl_3$).

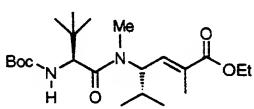
General procedure 1: Trifluoacetic acid mediated cleavage of *N*-Boc groups

N-Boc-amino acid ester (1.0 equiv.) was treated with TFA /CH_2Cl_2 (0.1 mmol / 1 mL) at room temperature for 0.5 h. Removal of the solvent *in vacuo*, followed by repeated rinsing of the residual material with CH_2Cl_2 (3 x 5 mL) and evaporation of the remaining traces of solvent afforded the TFA salt of the amino acid ester in quantitative yield. TFA salts were used without further purification.

General procedure 2: Trimethylacetyl chloride mediated peptide coupling

To a cold (-78 C) stirred solution of acid (1.1 equiv.) in dry THF (1 mL /mmol) under an argon atmosphere was added DIEA (1.5 equiv.) and trimethylacetyl chloride (1.2 equiv.). The resulting mixture was warmed to 0^{o}C for 1 h and then re-cooled to -78 C. DIEA (2.2 equiv.) was added *via* cannula to the reaction flask followed by the addition *via* cannula of the TFA salt of the amino acid ester (1.0 equiv., prepared by general procedure 1) in dry THF (0.5 mL/mmol) at -78 C. Stirring was continued for 1 h and water (40 mL) was added. The mixture was allowed to warm to room temperature, and extracted with diethyl ether (3 x 50 mL). The combined organic extracts were washed with saturated aqueous sodium chloride (50 mL), dried over magnesium sulfate, and concentrated *in vacuo*. The crude oil was purified by flash chromatography (silica gel, diethyl ether - pet. ether) to afford the desired dipeptide as a colourless oil.

Dipeptide 8



8

Following general procedure 2, dipeptide **8** was prepared with the following quantities of reagents and solvents: *N*-Boc-*tert*-leucine (**15**), 156 mg (0.52 mmol); trimethylacetyl chloride, 64 mL (0.52 mmol); DIEA, 99 mL (0.57 mmol); *N*-Boc-MHVV-OEt (**14**), 110 mg (0.47 mmol): DIEA, 198 mL (1.14 mmol); THF, 7 mL. Purification of the crude product by flash chromatography (silica gel, 1 : 5 diethyl ether - pet. ether) afforded 121 mg of **8** (62% yield). ^{1}H-NMR (200 MHz, $CDCl_3$) 0.76 (d, J = 6 Hz, 3H, CH_3), 0.80 (d, J = 6 Hz, 3H, CH_3), 0.88 (s,

9H, $(CH_3)_3$), 1.22 (t, J = 7 Hz, 3H, CH_3), 1.33 (s, 9H, Boc-$(CH_3)_3$), 1.79 - 1.89 (m, 1H, CH), 1.83 (s, 3H, CH_3), 2.91 (s, 3H, NCH_3), 4.12 (q, J = 7 Hz, 2H, CH_2), 4.35 (d, J = 10 Hz, 1H, CH), 5.03 (t, J = 10 Hz, 1H, CH), 5.14 (d, J = 10 Hz, 1H, NH), 6.57 (d, J = 8 Hz, 1H, CH); Exact mass calc'd for $C_{22}H_{41}N_2O_5$ $(M+H)^+$: 413.30154; Found (DCI): 413.30119. Optical rotation obtained was $[\alpha]_D^{25}$ -76.9 (c 2.43, $CHCl_3$).

Assay for Cytotoxicity

Cytotoxicity of SPA-110 compared to hemiasterlin as against p53+ and p53- variants of human breast cancer MCF-7 cells and A549 tumor cells was determined according to the methods described in J. Immunol. Methods 65:55-63 (1983). Results shown in Figures 5A and 5B show that SPA-110 is more cytotoxic in some instances than the naturally occurring compound.

Assay for Antimitotic Activity

Antimitotic activity is detected by enzyme-linked immunosorbent assay using a mitosis-specific antibody, TG-3 (from Albert Einstein College of Yeshiva University, Bronx, N.Y.; and see: PCT application published July 4, 1996 under WO96/20218).

MCF-7 mp53$^-$ cells (expressing a dominant-negative p53 mutation as described in S. Fan, et al. (1955) Cancer Research 55:1649-1654) were cultured as monolayers in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 units /ml penicillin, 50 μg/ml streptomycin, 1 mM sodium pyruvate, MEM non-essential amino acids, 1 μg/ml bovine insulin, 1 μg/ml hydrocortisone, 1 ng/ml human epidermal growth factor, and 1 ng/ml β-estradiol at 37°C in humidified 5% CO_2. The cells were seeded at 10,000 cells per well of 96-well polystyrene tissue culture plates (Falcon) in a volume of 200 μl cell culture medium. The cells were allowed to grow for 24 hours and compounds were added at about 1 μg/ml or 10 μg/ml (from 1000-fold stocks in dimethylsulfoxide) and the cells were incubated for 20 hours. Nocodazole (Sigma) served as a positive control. After treatment with the agent to be tested, the cell culture medium was removed completely and the 96-well tissue culture plates were frozen at -70°C for up to 2 hours. The frozen cells were thawed by addition of 100 μl

of ice-cold lysis buffer (0.5 mM phenylmethylsulfonylfluoride, 1 mM ethylene glycol-bis(β-aminoethyl ether) N,N,N,N'-tetraacetic acid, pH 7.4,) and lysed by pipeting up-and-down 10 times. The cell lysates were transferred to 96-well PolySorp ELISA plates (Nunc) and dried completely by blowing warm air at about 37°C with a hair drier positioned about 3 feet above the plates. Protein binding sites were blocked by adding 200 μl per well of 10 mM Tris HCl pH 7.4, 150 mM NaCl, 0.1 mM PMSF, 3% (w/v) dried non-fat milk (Carnation) for 1 hour at room temperature. This was removed and replaced with 100 μl of the same solution containing 0.1-0.15 μg/ ml TG-3 mitosis-specific monoclonal antibody and horseradish peroxidase-labelled goat anti-mouse IgM (1021-05, Southern Biotechnology Associates) at a dilution of 1/500. After overnight incubation at 4°C, the antibody solution was removed and the wells were rinsed 3 times with 200 μl rinse buffer (10 mM Tris HCl pH 7.4, 0.02% Tween 20). 100 μl of 120 mM Na_2HPO_4, 100 mM citric acid, pH 4.0 containing 0.5 mg/ml 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 0.01% hydrogen peroxide was added for 1 hour at room temperature and the plates were read at 405 nm using a BioTek plate reader.

Results comparing antimitotic activity of hemiasterlin to SPA-110 are shown in Figure 6. SPA-110 exhibited considerably greater antimitotic activity than the naturally occurring compound.

In Vivo Activity of SPA-110

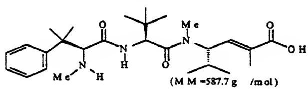
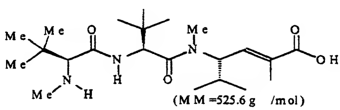
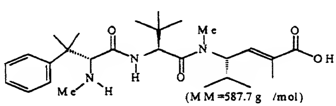
The compound SPA-110 was evaluated *in vivo* using standard pharmacological test procedures which measure its ability to inhibit the growth of human tumor xenografts. The human colon carcinoma LOVO (American Type Culture Collection, Rockville, Maryland #CCL-229) was grown in tissue culture in RPMI supplemented with 10% FBS. Athymic

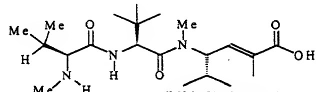
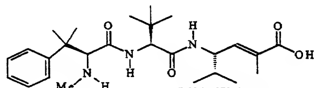
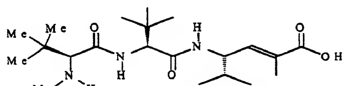
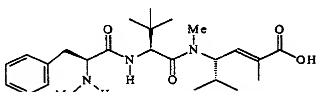
nu/nu female mice (Charles River, Wilmington, MA) were injected sub-cutaneously in the flank area with 7.0 x 10^6 LOVO cells. When tumors attained a mass of between 80 and 120 mg, the mice were randomized into treatment groups (day zero). Animals were treated intravenously once a day on days 1, 5, and 9 post staging (day zero) with 1 mg/kg/dose of SPA-110 prepared in 2.5% ethanol in saline or with saline as the vehicle control. Some animals were treated intraperitonealy once a day on days 1, 5 and 9 post staging with 1 mg/kg/dose Vincristine as a positive control. Tumor mass was determined every 7 days [(length x width^2)/2] for 28 days post staging. RTG or relative tumor growth (Mean tumor mass on days 7, 14, 21 and 28 divided by the mean tumor mass on day zero) was determined for each treatment group. % T/C was calculated as RTC (treated group) % RTC (vehicle control group) x 100. Statistical analysis (Student-t-test) of log relative tumor growth was used to compare treated verses control group in each experiment. A p-value ($p \leq 0.05$), which indicates a statistically significant reduction in relative tumor growth, was obtained in each case. 5 of 5 animals treated at day 28 with SPA-110 survived. 9 of 10 animals treated at day 28 with vincristine survived. The results are shown in the following table.

Treatment	% T/C day 7	% T/C day 14	% T/C day 21	% T/C day 28
Vincristine	34	37	44	47
SPA-110 1 mg/kg	16	18	30	47

Relative Activity of Compounds of this Invention

Various analogs of SPA-110 have been synthesized and characterized for their cytotoxic and anti-mitotic activities. The high degree of correlation between cytotoxicity and anti-mitotic capacity indicates that the cytotoxicity of the compounds of this invention is due to the compounds' anti-mitotic activity. The following structures are analogs falling within the scope of this invention depicted in approximately descending order of cytotoxic/anti-mitotic activity.

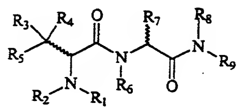
Compound Name	Compound structure (TFA salt) $Me = CH_3$
SPA 110	
SPA115	
SPA123	

SPA116	 (MM=511.6 g /mol)
SPA121	 (MM=573.7 g /mol)
SPA122	 (MM =511.6 g /mol)
SPA114	 (MM=559.6 g /mol)

As will be apparent to those skilled in the art in the light of the foregoing disclosure, many alterations and modifications are possible in the practice of this invention without departing from the spirit or scope thereof. Accordingly, the scope of the invention is to be construed in accordance with the substance defined by the claims herein, which substance includes obvious chemical equivalents of the compounds and methods set out in the claims.

WE CLAIM:

1. A compound or pharmaceutically acceptable salt thereof, having the formula



I

wherein,

R_1 and R_2 are independently selected from the group consisting of: H, R, and ArR-, and where at least one of R_1 and R_2 is R and neither are ArR-, R_1 and R_2 together may optionally be a three to seven member ring;

R_3 and R_4 are independently selected from the group consisting of: H, R, ArR-, and where at least one of R_3 and R_4 is R and neither are ArR- or Ar, R_3 and R_4 together may optionally be a three to seven member ring;

R_5 is selected from the group consisting of: H, R, ArR-, and Ar;

R_6 is selected from the group consisting of: H, R, and ArR-;

R_7 and R_8 are independently selected from the group consisting of: H, R, and ArR-; and

R_9 is: $Z\text{-}\overset{O}{\overset{\|}{C}}\text{-}Y\text{-}$;

and wherein,

R is defined as a saturated or unsaturated moiety having a linear, branched, or cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, -OH, $-OR_{10}$, $-O_2CR_{10}$, -SH, $-SR_{10}$, $-SOCR_{10}$, $-NH_2$, $-NHR_{10}$, $-N(R_{10})_2$, $-NHCOR_{10}$, $-NR_{10}COR_{10}$, -I, Br, -Cl, -F, -CN, $-CO_2H$, $-CO_2R_{10}$, -CHO, $-COR_{10}$, $-CONH_2$, $-CONHR_{10}$, $-CON(R_{10})_2$, -COSH, $-COSR_{10}$, $-NO_2$, $-SO_3H$, $-SOR_{10}$, $-SO_2R_{10}$, wherein R_{10} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

X is defined as a moiety selected from the group consisting of: -OH, -OR, =O, =S, $-O_2CR$, -SH, -SR, -SOCR, $-NH_2$, -NHR, $-N(R)_2$, -NHCOR, -NRCOR, -I, -Br, -Cl, -F, -CN, $-CO_2H$, $-CO_2R$, -CHO, -COR, $-CONH_2$, -CONHR, $-CON(R)_2$, -COSH, -COSR, $-NO_2$, $-SO_3H$, -SOR, and $-SO_2R$;

Ar is defined as an aromatic ring selected from the group consisting of: phenyl, naphthyl, anthracyl, phenanthryl, furyl, pyrrolyl, thiophenyl, benzofuryl, benzothiophenyl, quinolinyl, isoquinolyl, imidazolyl, thiazolyl, oxazolyl, and pyridinyl, optionally substituted with R or X;

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R, ArR-, or X; and,

Z is defined as a moiety selected from the group consisting of: -OH, -OR; -SH; -SR; $-NH_2$; -NHR; $-N(R)_2$; $-NHCH(R_{11})COOH$; and $-NRCH(R_{11})COOH$, wherein R_{11} is a moiety having the formula: R, or $-(CH_2)_nNR_{12}R_{13}$, wherein n=1-4 and R_{12} and R_{13} are independently selected from the group consisting of: H; R; and $-C(NH)(NH_2)$.

2. The compound of claim 1 wherein Ar is phenyl, naphthyl, anthracyl, or pyrrolyl.

3. The compound of claim 2 where R_5 is phenyl, naphthyl, anthracyl, or pyrrolyl.

4. The compound of claim 1, 2 or 3 wherein R_3 and R_4 are independently selected from the group consisting of: methyl, ethyl, n-propyl and n-butyl; or, R_3 and R_4 together are selected from the group consisting of: β-cyclopropyl, β-cyclobutyl, β-cyclopentyl and β-cyclohexyl.

5. The compound of any of claims 1-4 wherein R_1 and R_2 are independently selected from the group consisting of: H, methyl, ethyl, propyl, n-butyl, acetyl; or, R_1 and R_2 are joined and form a moiety selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

6. The compound of any of claims 1-4 wherein R_1 and R_2 are independently: H, CH_3 or acetyl.

7. The compound of any of claims 1-4 wherein R_1 is H, and R_2 is $-CH_3$.

8. The compound of any of claims 1-7 wherein Z is: OH, $-OCH_3$, $-NHCH(R_{11})COOH$, or, $-NCH_3CH(R_{11})COOH$, wherein R_{11} is R, or $-(CH_2)_nNHC(NH)(NH_2)$.

9. The compound of any of claims 1-7 wherein Z is OH or $-OR_{14}$, wherein R_{14} is a linear or branched one to six carbon alkyl group.

10. The compound of any of claims 1-7 wherein R_9 has the formula:

$$-\underset{R_{15}}{C}-C=C(R_{16})\overset{O}{\overset{\|}{C}}-OH$$

wherein R_{15} is selected from the group consisting of: methyl, ethyl, n-propyl, isopropyl, tert-butyl, iso-butyl, and sec-butyl; and R_{16} is selected from the group consisting of: H, methyl, ethyl, propyl, iso-propyl, n-butyl, iso-butyl and sec-butyl.

11. The compound of claim 10 wherein R_{15} is isopropyl and R_{16} is methyl.

12. The compound of any of claims 1-11 wherein R_7 is a three to six carbon, branched alkyl group.

13. The compound of any of claims 1-12 wherein R_6 and R_8 are independently: H, or CH_3.

14. The compound of any of claims 1-11 wherein R_6 is H, R_7 is: $-C(CH)_3)_3$, and R_8 is $-CH_3$.

15. The compound of any of claims 1-14 wherein R_3 and R_4 are each R.

16. The compound of any of claims 1-14 wherein R_3 and R_4 are each $-CH_3$.

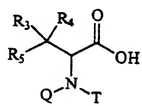
17. The compound of claim 16 wherein R_5 is phenyl.

18. The compound of claim 17 wherein R_9 has the formula:

$$-\underset{CH(CH_3)_2}{C}-C=C(CH_3)\overset{O}{\overset{\|}{C}}-OH$$

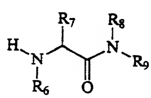
19. A method of preparing a compound as described in claim 1 comprising the step of:

(a) coupling an amino acid having the formula:



in which R_3 - R_5 are as defined in claim 1 and Q and T are selected from the group consisting of: R_1 and R_2 as defined in claim 1, and a protecting group;

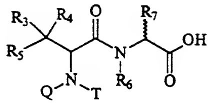
with a dipeptide having the formula:



in which R_6 - R_9 are as defined in claim 1;

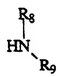
and, where Q or T is a protecting group, the additional step of replacing the protecting group with R_1 or R_2 to form compound I; or,

(b) coupling a dipeptide having the formula:



in which R_3 - R_7 are as defined in claim 1 and Q and T are as defined above;

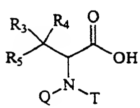
with an amino acid having the formula:



in which R_8 and R_9 are as defined in claim 1;

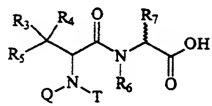
and, where Q or T is a protecting group, the additional step of replacing the protecting group with R_1 or R_2 to form compound I.

20. An amino acid suitable for use in the method of claim 19, having the formula:



in which R_3 - R_5, Q and T are as defined in claim 19.

21. A dipeptide suitable for use in the method of claim 19, having the formula:



in which R_3 - R_7, Q and T are as defined in claim 19.

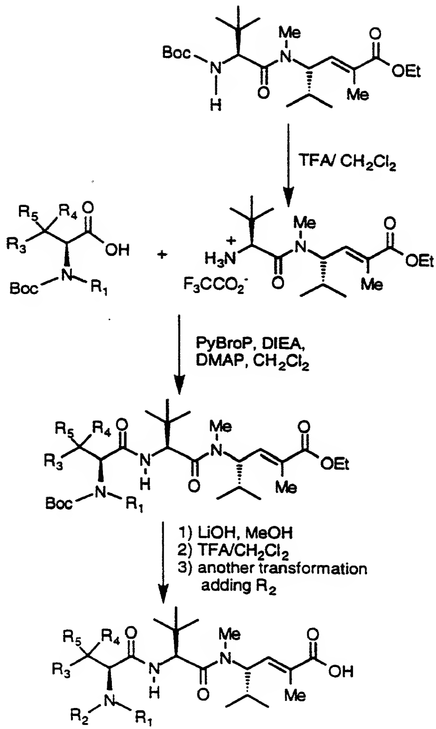

TFA/ CH2Cl2
PyBroP, DIEA,
DMAP, CH2Cl2
1) LiOH, MeOH
2) TFA/CH2Cl2
3) another transformation
adding R2

Figure 1

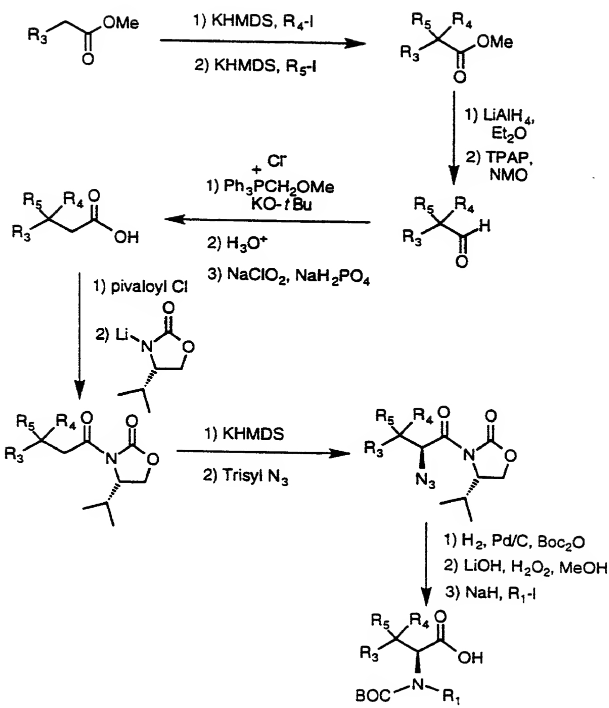

OMe
R_3
O
1) KHMDS, R_4-I
2) KHMDS, R_5-I
R_5 R_4
OMe
R_3
O
1) $LiAlH_4$, Et_2O
2) TPAP, NMO
Cl^-
1) $Ph_3\overset{+}{P}CH_2OMe$
KO-tBu
2) H_3O^+
3) $NaClO_2$, NaH_2PO_4
R_5 R_4 O
R_3
OH
R_5 R_4
H
R_3
O
1) pivaloyl Cl
2) Li
N
O
O
R_5 R_4 O O
R_3
N
O
1) KHMDS
2) Trisyl N_3
R_5 R_4 O O
R_3
N
O
N_3
1) H_2, Pd/C, Boc_2O
2) LiOH, H_2O_2, MeOH
3) NaH, R_1-I
R_5 R_4 O
R_3
OH
BOC
N
R_1

Figure 2

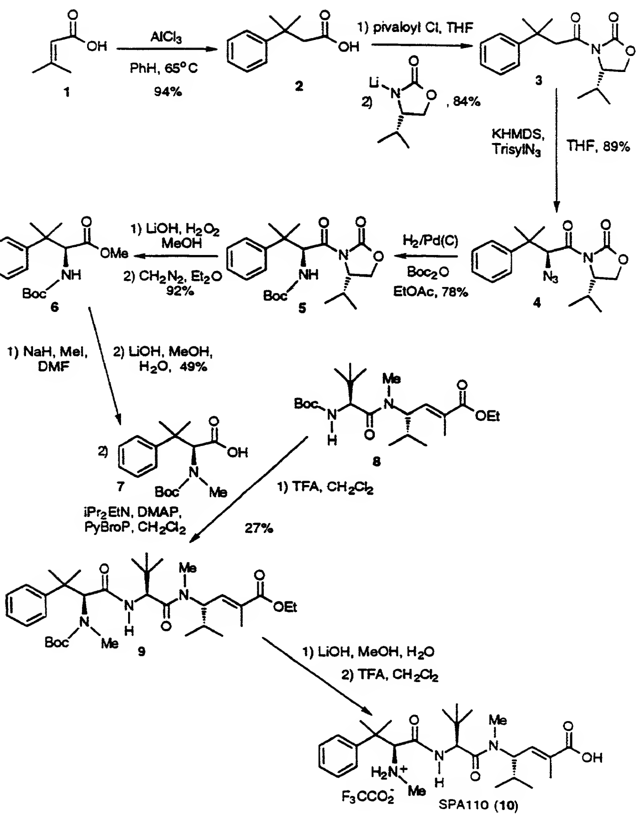
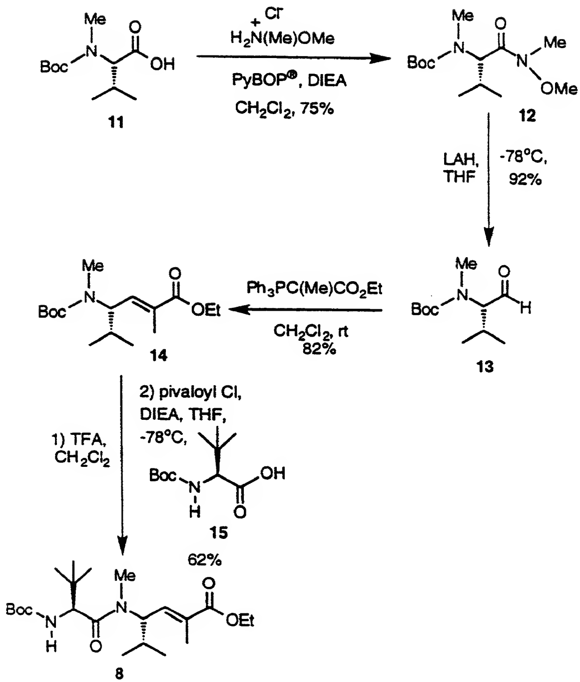


Figure 3



Me
O
Boc
N
OH
11
Cl⁻
+
H2N(Me)OMe
PyBOP®, DIEA
CH2Cl2, 75%
Me
O
Me
Boc
N
N
OMe
12
LAH,
THF
-78°C,
92%
Me
O
Boc
N
H
13
Ph3PC(Me)CO2Et
CH2Cl2, rt
82%
Me
O
Boc
N
OEt
14
1) TFA,
CH2Cl2
2) pivaloyl Cl,
DIEA, THF,
-78°C,
Boc
N
H
OH
O
15
62%
Me
O
Boc
N
H
O
N
OEt
8

Figure 4

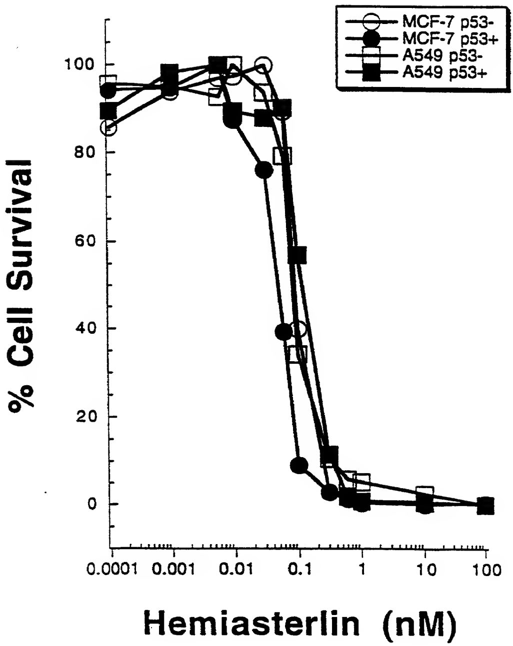

MCF-7 p53-
MCF-7 p53+
A549 p53-
A549 p53+
% Cell Survival
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
Hemiasterlin (nM)

FIGURE 5A

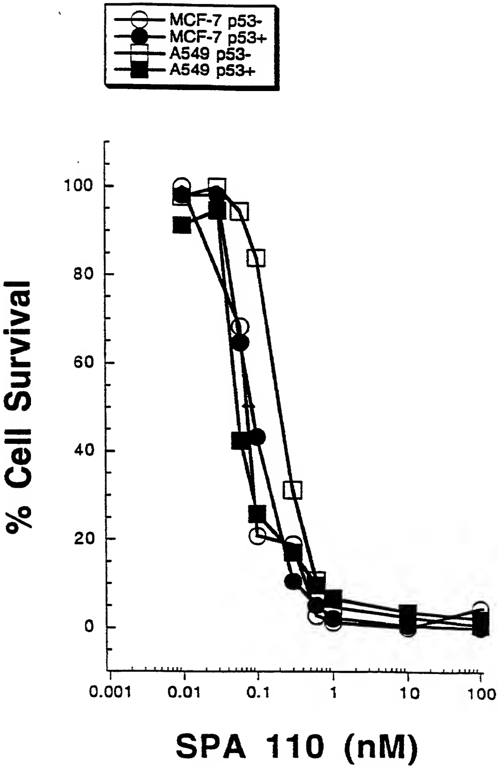

MCF-7 p53-
MCF-7 p53+
A549 p53-
A549 p53+
100
80
60
40
20
0
% Cell Survival
0.001
0.01
0.1
1
10
100
SPA 110 (nM)

FIGURE 5B

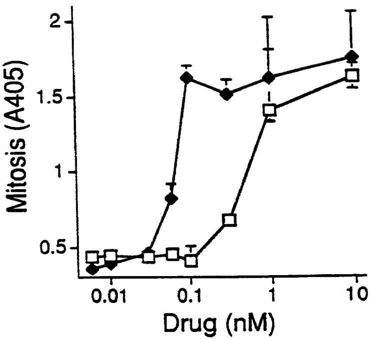


Figure 6

A, F, K, P & K Docket No. P108281-00000 **ARENT FOX KINTNER PLOTKIN & KAHN PLLC**

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
(Insert Title) HEMIASTERLIN ANALOGS
the specification of which is attached hereto unless the following box is checked:

☒ was filed on December 18, 1998 as PCT International Application Number PCT/CA98/01184
__________________.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

(List prior foreign applications. See note A on back of this page)				Priority Claimed
	2,225,325 (Number)	CA (Country)	19 December 1997 (Day/Month/Year Filed)	☒ Yes ☐ No
	(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
	(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

(See Note B on back of this page) ☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(List prior U.S. Applications or PCT International applications designating the U.S.)			
	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

And I hereby appoint as principal attorneys: Robert B. Murray, Reg. No. 22,980; David T. Nikaido, Reg. No. 22,663; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Douglas H. Goldhush, Reg. No. 33,125; Monica Chin Kitts, Reg. No. 36,105; Richard J. Berman, Reg. No. 39,107; King L. Wong, Reg. No. 37,500; James A. Poulos, III, Reg. No. 31,714; Murat Ozgu, Reg. No. 44,275; Bradley D. Goldizen, Reg. No. 43,637; N. Alexander Nolte, Reg. No. 45,689; Robert K. Carpenter, Reg. No. 34,794; Gregory B. Kang, Reg. No. 45,273; and Rustan J. Hill, Reg. No. 37,351.

Please direct all communications to the following address: Arent Fox Kintner Plotkin & Kahn PLLC
1050 Connecticut Avenue, N.W., Suite 600
Washington, D.C. 20036-5339
Telephone (202) 857-6000; Telefax (202) 857-6395

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note C on back of this page)

Full name of sole or first inventor: Raymond ANDERSEN
Inventor's signature ________ Aug 16/2000 Date

100

Residence: 4048 West 32nd Avenue, Vancouver, British Columbia V6S 1Z6, Canada CAX
Citizenship: Canada
Post Office Address: Same as above

Rev. #15 1/14/00

Full name of second joint inventor, if any: Edward PIERS

Inventor's signature ______________________ ______________ Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of third joint inventor, if any: James NIEMAN

Inventor's signature ______________________ ______________ Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of fourth joint inventor, if any: John COLEMAN

Inventor's signature ______________________ ______________ Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of fifth joint inventor, if any: Michel ROBERGE

Inventor's signature M. Roberge 16 AUGUST 2000 Date

Residence: 4228 West 10th Avenue, Vancouver, British Columbia V6R 2H4, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of second joint inventor, if any: Edward PIERS

Inventor's signature ______________________ ______________ Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of third joint inventor, if any: James NIEMAN

Inventor's signature ______________________ ______________ Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of fourth joint inventor, if any: John COLEMAN

Inventor's signature J Coleman Aug 21, 00 Date

Residence: ~~c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada~~ OCEAN NUTRITION CANADA, 1721 LOWER WATER ST, HALIFAX, N.S. B3J 1S5 Aug 21, 00

Citizenship: Canada CAX

Post Office Address: Same as above

Full name of fifth joint inventor, if any: Michel ROBERGE

Inventor's signature M. Roberge 16 AUGUST 2000 Date

Residence: 4228 West 10th Avenue, Vancouver, British Columbia V6R 2H4, Canada

Citizenship: Canada CAX

Post Office Address: Same as above

Full name of second joint inventor, if any: Edward PIERS

Inventor's signature ______________________ ______________ Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

300

Full name of third joint inventor, ~~if any~~: James NIEMAN

Inventor's signature ______________________ August 18, 2000 Date

Residence: ~~c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada~~ 357 Lake Forest Blvd., ~~Kalamazoo, MI~~, US, 49006 MI JN

Citizenship: Canada

Post Office Address: Same as above

Full name of fourth joint inventor, if any: John COLEMAN

Inventor's signature ______________________ ______________ Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of fifth joint inventor, if any: Michel ROBERGE

Inventor's signature M. Roberge 16 AUGUST 2000 Date

Residence: 4228 West 10th Avenue, Vancouver, British Columbia V6R 2H4, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of second joint inventor, if any: Edward PIERS

Inventor's signature Edward Piers Sept. 7, 2000
Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada CAY

Post Office Address: Same as above

Full name of third joint inventor, if any: James NIEMAN

Inventor's signature
Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of fourth joint inventor, if any: John COLEMAN

Inventor's signature
Date

Residence: c/o The University of British Columbia, Dept. of Chemistry, 6328 Memorial Road, Vancouver, British Columbia V6T 1Z3, Canada

Citizenship: Canada

Post Office Address: Same as above

Full name of fifth joint inventor, if any: Michel ROBERGE

Inventor's signature M. Roberge 16 AUGUST 2000
Date

Residence: 4228 West 10th Avenue, Vancouver, British Columbia V6R 2H4, Canada

Citizenship: Canada

Post Office Address: Same as above